THE AXIAL GRADIENT IN CILIATE INFUSORIA.

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The discovery of axial gradients in rate of metabolic reactions in *Planaria* (Child '12, '13*a*, '13*c*) and various other forms together with a considerable body of evidence, mostly as yet unpublished, which points to a close relation between such axial gradients and polarity and symmetry suggested the desirability of determining whether there is any indication of the existence of such axial gradients in those protozoa which possess a definite morphological polarity. If an axial gradient in rate of metabolic reactions is the basis of organic polarity or is in any way associated with it we must expect to find such a gradient in single cells where a definite stable polarity exists as well as in multicellular axiate organisms. The results obtained with various ciliates have fully justified this expectation. In all the forms tested thus far evidence for the existence of an axial gradient has been found.

I. Methods.

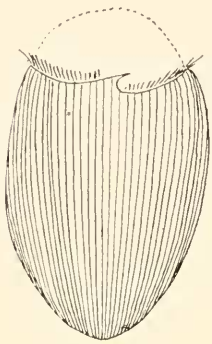
In these experiments the method which I have called the direct susceptibility or resistance method (Child, '13*b*) was used, with KCN as the reagent in most cases. This method is based upon the fact determined by extensive experimentation, that in concentrations of the cyanides and various anesthetics which kill within at most a few hours, individuals or regions with a higher rate of metabolism or at least of the oxidation processes are more susceptible and die earlier than those with a lower rate.

In the experiments with ciliates concentrations were found which did not kill the animals at once but permitted life to continue for a period ranging from a few moments up to several hours according to the concentration. The purpose in thus limiting the concentrations used is first to avoid killing the animals too rapidly, in which case the death and disintegration of all parts may be so nearly simultaneous that a gradient if present will be scarcely or not at all apparent. If on the other hand the concentration is too low, the results may be complicated by acclimation, the individuals or regions of highest rate showing in general the greatest capacity for acclimation. The proper concentrations for each species must of course be determined by experiment.

II. Heterotricha.

The species *Stentor cœruleus* has been the chief experimental material from this group. In KCN the animals do not become elongated and attach themselves but contract to a rounded form and swim continuously backward by means of the cilia. In KCN 0.002 *m*. at a water-temperature of 25°–26° C. all animals (several hundred in each experiment) remain alive and intact and swim continuously during the first hour. At the end of this time a very few individuals show the first stages of disintegration. From this time on death and disintegration occur until at the end of the second hour all are dead. At lower temperatures the survival time is longer.

The usual course of death and disintegration is indicated in Figs. 1–5. In each figure the disintegrated portion is indicated merely by a dotted outline. While the animal is swimming about the peristome region or some part of it, often a region near the mouth first, begins to swell and bulge. This change spreads over the whole peristome within a few seconds or in many individuals is practically simultaneous in all regions of the peristome (Fig. 1). This change is accompanied by complete disappearance of the ectoplasmic striations and other structure in the region involved and the entoplasm slowly swells and spreads out. Within two or three seconds after the first indications of change a halo of blue pigment appears in the water about the disintegrated region which has now lost most of its blue color. After a short pause varying in length in different individuals from twenty or thirty seconds to two or three minutes the disintegration extends beyond the peristomal cilia and begins its course toward the aboral end of the body (Fig. 2), obliterating the morphological structure of the cytoplasm as it proceeds. The boundary between the disintegrated and intact region is always sharp and clear and the course of disintegration can be followed without the slightest difficulty. The change at any given level is almost instantaneous: the structure seems simply to dissolve, the cytoplasm swells and the halo of blue pigment appears in the water while the disintegrated part loses most of its color. The peristomal cilia continue to beat until the instant



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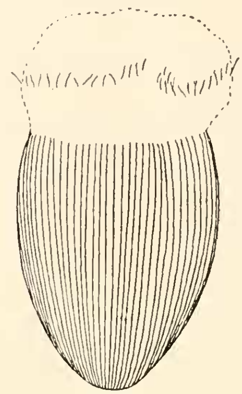

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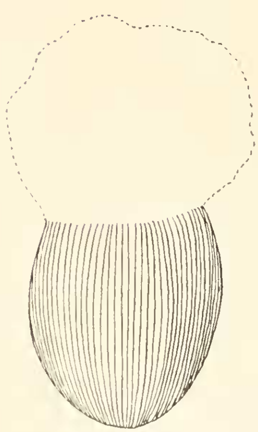

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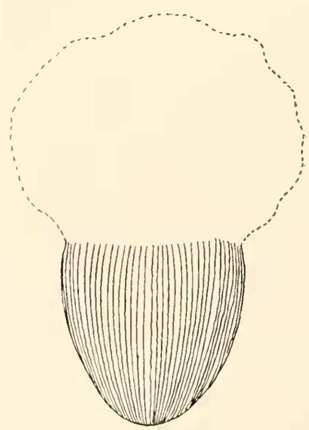

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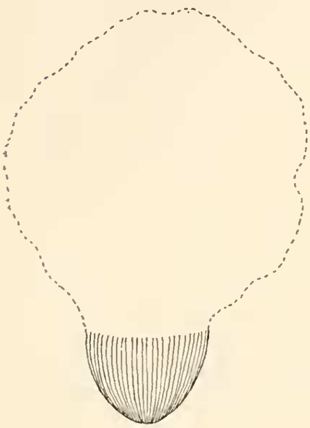
when the boundary of the spreading areas of disintegration reaches them when they suddenly stop in most cases, although occasionally they may beat for a few seconds more, but in all cases they disintegrate very soon. As soon as the peristomal cilia cease their movement the body ceases to revolve in the water, but the smaller cilia covering the body continue to beat until the disintegration wave reaches them. From the peristome region the process of disintegration spreads downward over the body as indicated in Figs. 2–5, the aboral end



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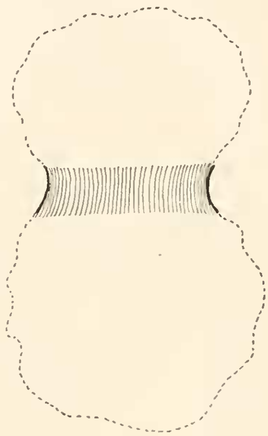

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being the last region to lose its structure. The disintegration of the aboral half is in practically all cases much more rapid than that of the oral half; very commonly the disintegration of the oral half requires two to four minutes, that of the aboral half one to two minutes, *i. e.*, the differences in susceptibility beween different levels are much greater in the anterior than in the posterior half. Moreover, the anterior half and particularly the peristome region lose their pigment more completely and swell and spread out to a greater extent in disintegration than the posterior half. Finally the animal is represented by a shapeless mass of protoplasm in which the meganucleus remains intact for a long time and about which a halo of blue pigment appears. With the concentration and temperature above mentioned the whole process of death and disintegration requires from two to six minutes, the rate of advance of the disintegration varying in different individuals and in some cases in different regions of the body.

The striking features of the change are its very definite course, the distinct boundary between disintegrated and intact regions at all times and the uniformity with which the change advances at all points of the circumference of the body. Occasionally, however, modifications of the process appear. In a small percentage of the animals used for the experiment, not more than one or two per cent., the advance of disintegration ceases for a time when it has reached about the stage represented in Fig. 3, *i. e.*, when about the oral half is disintegrated. Then after one or two minutes the disintegration of the aboral half occurs almost simultaneously in all parts. In one individual observed (Fig. 6) the disintegration of the oral half occurred in the usual manner, then there was a pause of about one minute and then the disintegration of the aboral half began from the aboral end. Fig. 6 shows this individual a few seconds before disintegration was complete. A narrow band of intact body wall on which the cilia were seen to be still moving separates the two areas of disintegration. During the next few seconds the aboral area advanced over this band and completed the process. This is the only case among thousands observed in which disintegration proceeded from the aboral end instead of toward it.

The length of life in the reagent before disintegration begins and the rate of advance of disintegration over the body vary within wide limits according to water-temperature, concentration of the reagent and alkalinity, but the course of the process is the same in all cases with the rare modifications described above.

Another species of *Stentor*, apparently *S. polymorphus*, gives the same results, but the course of disintegration is not so readily followed in this species because of the absence of pigment and the less conspicuous myonemes.

III. Hypotricha.

Several species of hypotrichous ciliates including two species of *Stylonychia*, *Euplotes* and two unidentified forms have thus far been tested by this method and with the same result in all cases, viz., death and disintegration begin at the anterior end and proceed down over the body to the posterior end. When the animals are placed in KCN they at once begin to swim backward and continue to do so until death and disintegration occur.

The process of disintegration is in all essential features similar to that in *Stentor* and consists in a dissolution of the structure and a swelling of the cytoplasm. The boundary between the disintegrated and intact regions is sharp and the disintegrative change proceeds uniformly down the body, the rate of advance varying in different individuals and with different temperatures and concentrations of reagent.

In *Stylonychia* the beginning of disintegration is marked by the melting down of the structures at the anterior end into a mass of uniform semi-fluid cytoplasm while the cilia elsewhere are still active and the animal is moving rapidly backward. The dissolution of structure extends backward over the body and the disintegrated mass often stretches out and is left behind in a long trail of granules and semifluid droplets as the animal moves backward. Movement continues in each part until the advancing wave of disintegration reaches it, when it suddenly breaks down and seems to dissolve into a formless mass. The posterior end continues to move until finally it too dissolves and movement ceases.

The advance of the disintegration wave and the sudden melting of each structure as the wave reaches it are striking phenomena and the uniformity of the whole process in different individuals and in different species indicates that it is associated with a fundamental feature of the constitution of these cells.

If the concentration of the cyanide is so low that the animals live for an hour or more before disintegration begins *Stylonychia* usually loses its flattened elongated form and becomes almost spherical, but movement still continues and the anterior end is still distinguishable by its structure. In such cases the disintegration, when it finally occurs, is more rapid and extends from anterior to posterior end in a few seconds.

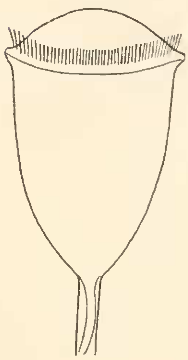
IV. Peritricha.

The forms examined include three species of Vorticellidæ and one of *Carchesium*. The results are practically identical in all cases. All of these forms are more resistant than *Stentor* and require a higher concentration of reagent. In *Carchesium* for example the course of death and disintegration in KCN

0.01 *m*. is as follows: The first change is a bulging of the peristome (Fig. 7) as in *Stentor*, but in these forms this does not usually occur until after the cilia have ceased or almost ceased to beat. This bulging is followed within a few seconds by disintegration of the peristome region including the cilia (Fig. 8), while the rest of the body is still intact and then a pause of a few seconds occurs. Disintegration of the body below the peristome is much more rapid than in *Stentor* and during the next few seconds the structure, except for the meganucleus, disappears completely and the entoplasm swells and spreads out into the water, the process beginning at the oral region and rapidly advancing aborally. But the ectoplasm does not disintegrate completely: a part of the body wall is elastic and as the contents of the body flow out this structure contracts and remains visible for a long time as a transparent skeleton or "shadow" of the body, much reduced in size but retaining more or less the original shape (Figs. 9 and 10). So far as can be determined the ectoplasm of the peristome region does not take part in the contraction but after the early stages of disintegration disappears completely. Evidently the lateral body wall is differentiated in part into a supporting substance which is no longer metabolically active. In this connection the difference in contractility between the peristome region and the lateral walls of the body in the living animal is of interest. The peristome is highly contractile and can be closed and opened while other parts of the body wall are capable of but little contraction. The contractile region disintegrates completely at once in KCN while the non-contractile region does not.

The stalk resembles the lateral body wall in its resistance but the contractile fiber usually shows disintegrative changes at about the same time as the peristome region or a little later. These changes consist in a loss of the regular contour, the appearance of varicosities and an apparent breaking up into a series of granules or droplets. Examination of the fiber with very high powers has not been made.

When lower concentrations, *e. g.*, KCN 0.002 *m*. are used, the death changes are limited to the appearance of hyaline or slightly granular droplets on the peristome region (Fig. 11) or to a bulging of the whole peristome (Fig. 12) and the body may remain otherwise intact for several days. The changes in ethyl ether 2 per cent. are very similar. In a small vorticellid of which only a few individuals were present in my cultures the death changes consisted of the bulging of the peristome (Fig. 13) and later of



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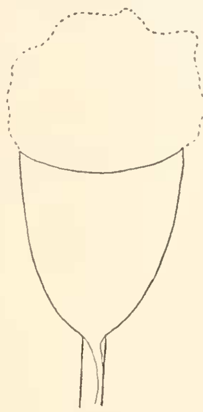

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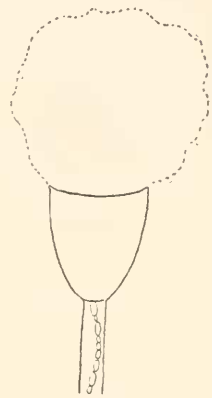

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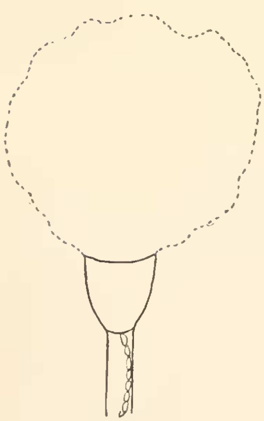

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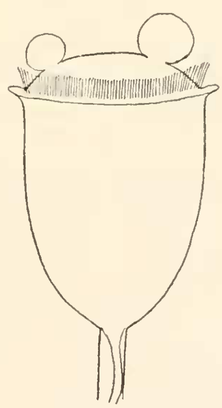

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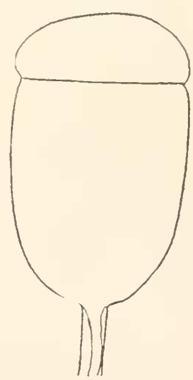

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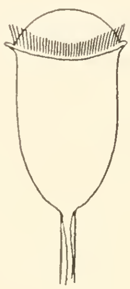
the disappearance of the cilia and the swelling of the cytoplasm until it formed a large rounded mass still enclosed within the stretched membrane of the peristome region (Fig. 14) while the lateral body-wall retained its form. In higher concentrations the changes in this form would undoubtedly be the same as in *Carchesium*.

In all four species examined the peristome region is very evidently more susceptible to the reagent than other parts. In the remainder of the body the death changes do progress from the oral to the aboral end, but their progress is much more rapid than in *Stentor*. In KCN 0.01 *m.* disintegration is completed within 5–10 seconds after the pause following peristome disintegration. In these forms then the axial gradient consists first



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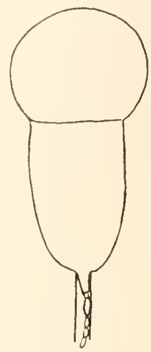

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in a marked decrease in susceptibility from the peristome region to more aboral regions and a slight decrease in the rest of the body from the oral to the aboral end.

V. Holotricha.

Paramœcium is not very susceptible to KCN, but the individual differences in resistance are considerable. In KCN 0.01 *m.* with water-temperature 22°–25° C. average survival time of animals from one culture was six or seven hours, but some animals die after two or three hours and some live eight or ten hours. Moreover, different cultures differ very considerably in resistance to cyanide.

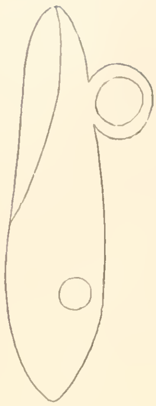
When the animals are placed in KCN they at once begin to swim backward. This continues for a period varying from a few minutes to half an hour or in some cases longer. Gradually the backward movement becomes less and less marked until the animal is simply revolving and then sooner or later it begins to swim forward once more and continues until it dies.

In KCN 0.01 *m.* the disintegration changes begin in the great majority of cases with the appearance of a rounded swelling over the anterior vacuole. The ectoplasm involved loses its cilia and its characteristic structure but a thin membrane remains surrounding the swelling. The swelling gradually increases in size and becomes a spherical mass into which the anterior vacuole often passes (Fig. 15). Here the vacuole undergoes enlargement and does not contract rhythmically but finally disappears. The vacuole is surrounded by granular cytoplasm. In other cases the anterior vacuole remains in its normal position and the swelling contains only granular cytoplasm (Fig. 16). In all cases the swelling is lighter in color than other parts of the body and the characteristic structure and the cilia of the region have disappeared.

Occasionally the swelling appears over the posterior instead of the anterior vacuole (Fig. 17) but this is comparatively a rare occurrence. In a few individuals among the thousands observed swellings of about equal size appeared over both vacuoles (Fig. 18) and in a few other cases a larger swelling over the anterior, a smaller over the posterior vacuole. But in at least nine out of ten the swelling appeared only on the anterior vacuole.

The swelling gradually enlarges, while the animal continues to swim, the vacuole disappears sooner or later (Fig. 19) and the intact portion of the body gradually decreases in size, the ectoplasm evidently undergoing contraction as the entoplasm gradually passes into the swelling (Fig. 20). At this stage the animal is still swimming, the structure of the ectoplasmic regions not involved in the swelling is intact and meganucleus and posterior vacuole retain their positions. As noted above the cytoplasm in the swelling, which is now as large as the remainder of the body, is lighter in color and more transparent and more uniform in appearance than in the intact regions. It is evidently dead and some of its constituents have probably diffused through the membrane as does the blue pigment in the case of *Stentor*.

The disintegrative change gradually spreads over the ectoplasm, less rapidly on the side of the oral groove than on the opposite side, and the intact ectoplasm continues to decrease in size (Fig. 21). The cilia on the intact portion are still present and beating at this stage and the animal may move slowly in a circular course with



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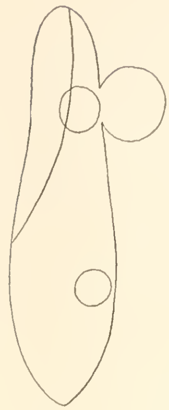

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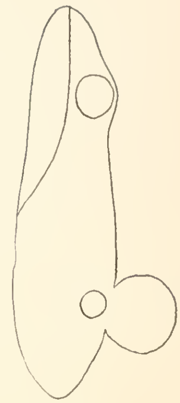

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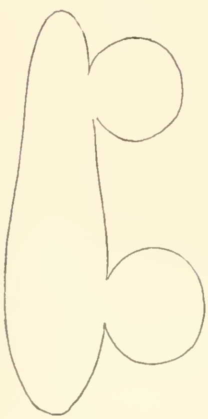

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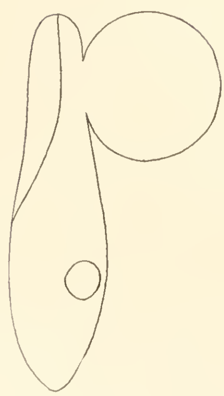

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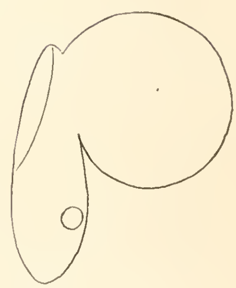

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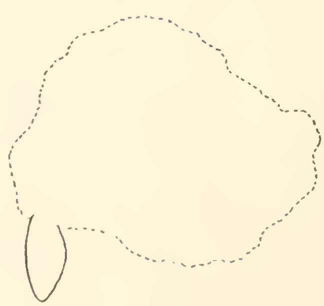

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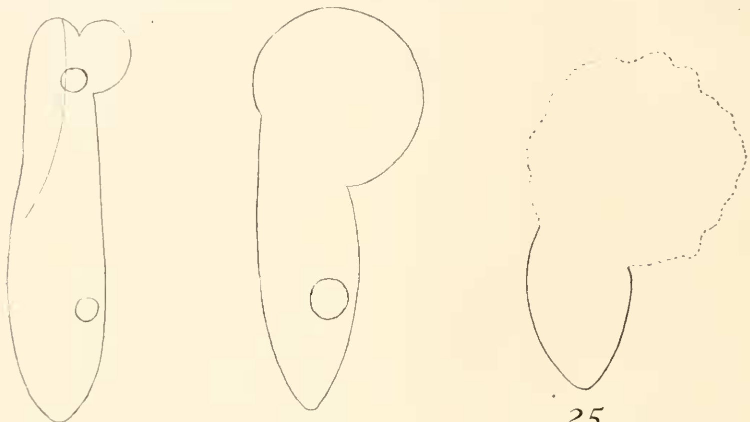
the swelling always toward the center. This direction of movement results from the distribution of the cilia and the weight of the swelling. In many cases the swelling bursts sooner or later, the contained cytoplasm flows out, a wave of disintegration accompanied by an increase in translucency passes rapidly over the remaining portions of the body, toward the posterior end, blotting out the ectoplasmic structure and the cilia and all that remains is the mass of granules spreading in the water, the meganucleus and a small transparent ectoplasmic "shadow" of the portions not directly involved in the swelling (Fig. 22).

In other cases the dissolution of structure occurs throughout the body before the rupture of the membrane and the body may retain the shape of Fig. 21 for some minutes but finally the membrane disappears and only a shapeless granular mass containing the meganucleus remains.

In higher concentrations of KCN or in lower concentrations with increased alkalinity disintegration is more rapid but its course is essentially the same. In KCN 0.02 *m.* or in KCN 0.001 *m.* which is made more alkaline by the addition of NaOH death and disintegration occur within ten to fifteen minutes. The course of disintegration is much the same as that described above, a small swelling containing granular cytoplasm lighter in color than the rest appears over the anterior vacuole, but the vacuole does not enter it in most cases (Fig. 23); this swelling increases rapidly in size and soon involves the anterior end of the body (Fig. 24); sooner or later its membrane disappears, the wave of disintegration spreads over the body, blotting out its structure and only a mass of granular protoplasm and the contracted "shadow" remaining (Fig. 25). In a few individuals droplets of semi-fluid hyaline substance appeared on other parts of the body surface and increased in size until bursting occurred.

In these solutions a swelling over the posterior vacuole appears much more frequently than in KCN 0.001 *m.* without the addition of alkali or even in 0.01 *m.* In most cases where a posterior swelling appears it is smaller than the anterior, but occasionally it is larger and rarely only the posterior swelling appears. In general, the higher the concentration of the reagent, whether with or without the addition of alkali, the more frequently the posterior as well as the anterior vacuole region swells. Increase in alkalinity accomplishes the same result as increase in concentration of KCN, *i. e.*, it increases susceptibility. In other words, when the concentration is so low that the animals die very slowly only the anterior vacuole region undergoes swelling before the death and disintegration of the rest of the body.

In still higher concentrations, *e. g.*, KCN 0.04 *m.* the animals die very soon, hyaline droplets appear on all parts of the body



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and in a few seconds nothing remains but the entoplasmic granules and the meganucleus more or less surrounded by the semi-fluid hyaline substance in rounded masses or droplets, apparently with a surface membrane. Sooner or later the droplets burst, apparently in consequence of rupture of the membrane, and the substance of the body spreads through the water. In such cases a gradient does not usually appear because the concentration is sufficiently high to kill all parts at the same time or nearly so.

Discussion.

It is evident from the preceding sections that a more or less definite axial gradient in susceptibility to KCN exists in all of the forms examined. These forms represent the four orders of ciliate infusoria, Heterotricha (*Stentor*), Hypotricha (*Stylonychia*, *Euplotes*, etc.), Peritricha (*Vorticella* and *Carchesium*) and Holotricha (*Paramœcium*), so that the existence of an axial gradient is evidently characteristic of the group.

If the susceptibility is in any degree a measure of the rate of metabolism or more specifically of oxidation processes as it unquestionably is in many other cases (Child 13*b*) we must conclude that an axial gradient in the rate of these processes is a characteristic feature of the ciliates. Moreover, the structure and behavior of the animals also suggest differences in rate of metabolic processes along the axis. In *Stentor* the peristome region is undoubtedly the most sensitive, most highly contractile and most active region of the body. In *Vorticella* and *Carchesium* a similar difference exists between peristome and body exclusive of the contractile fiber of the stalk. In *Stylonychia* the development and activity of cilia are greater in the anterior region than elsewhere. In the case of *Paramœcium*, however, the structure and behavior do not afford any definite evidence upon this point.

In forms like *Stentor, Vorticella* and *Carchesium*, where a special contractile peristome region is present, this region is distinguished from other regions of the body by a considerably greater degree of susceptibility, while in *Stylonychia* the gradient of susceptibility is almost uniform. Moreover in the elongated body of *Stentor* the gradient is steeper in the anterior than in the posterior half, *i. e.*, in the region which forms the stalk the gradient is relatively slight as compared with the body proper. In short there can, I think, be no doubt that the differences in susceptibility along the axis in *Stentor*, *Stylonychia*, *Euplotes*, *Vorticella* and *Carchesium* are an expression of a dynamic axial gradient, a gradient in the rate of certain fundamental metabolic processes.

The case of *Paramœcium* requires some further consideration. The constant association of the swelling with the vacuoles, and in the great majority of cases with the anterior vacuole alone raises the question whether we are to regard this region as possessing a higher rate of metabolism or of respiration than other parts of the body or whether some complicating factor, *e. g.*, weakness of the ectoplasmic wall in this region, plays a part in the localization of the swelling. There is no visible indication that the ectoplasm of the vacuolar region is thinner or structurally different in any way from that of other regions of the body, but such negative evidence is of course not conclusive. On the other hand the fact that the vacuole remains in its normal position at least as often as it enters the swelling indicates that the swelling is not simply the result of the enlargement of the vacuole and consequently stretching of the body wall. It should also be noted that when the swelling appears the ectoplasm does not merely stretch, it loses its structural features, including the cilia and the entoplasm which the swelling contains differs in appearance from that in other parts of the body. And finally, the vacuolar mechanism is very sensitive to cyanide, the pulsation ceases soon after the animal is placed in the solution and the vacuole slowly undergoes enlargement and sooner or later collapses and disappears. The vacuole region is apparently a specialized contractile mechanism and is undoubtedly the seat of a relatively high rate of dynamic activity. In short the only logical conclusion is that the greater susceptibility of the region where the swelling appears results from greater dynamic activity.

But a difference of some sort must exist between anterior and posterior vacuolar regions, for it was pointed out above (p. 47) that in the lower concentrations of the reagent only the anterior vacuolar region shows a swelling and that the frequency of swelling in the posterior as well as in the anterior vacuolar region increases as the concentration increases. Evidently the posterior vacuolar region is for some reason less susceptible than the anterior and a higher concentration is necessary at least in the great majority of individuals, to bring about swelling in it than in the anterior region.

A comparison of the rate of pulsation in anterior and posterior vacuoles affords a clue to the nature of this difference. I have found that in general the rate of the anterior vacuole is considerably higher than that of the posterior. Sometimes the ratio is as high as 5 : 3, in other cases less, but the difference in rate exists, at least in the great majority of individuals. Evidently the posterior vacuole has a lower rate of dynamic activity than the anterior, and the difference in susceptibility of the two vacuolar regions is undoubtedly associated with this difference in rate. In the lower concentrations or in individuals with a low susceptibility the special susceptibility appears as a rule only in the anterior vacuolar region because only here is the rate of reaction sufficiently high to bring about the rapid change. In higher concentrations the special susceptibility may appear in both vacuolar regions, but even in such cases the swelling usually appears earlier or is of larger size in the anterior region. In short the facts indicate that the two vacuolar regions of *Paramœcium* are more or less definitely localized regions of relatively high rate of metabolism or more specifically of respiration and, furthermore, that the rate in the anterior vacuolar region is higher than that in the posterior.

The lower susceptibility of the posterior vacuolar region to KCN and the lower rate of pulsation of the posterior vacuole in normal living animals suggest the existence of an axial gradient in rate of metabolism, and in the death and disintegration of the body in general this gradient appears as has been shown. When the disintegrative change begins to spread from the vacuolar region the anterior end of the body is attacked first and from this end the process of disintegration spreads downward to the posterior end. The differences in susceptibility along the axis are not as great as the differences between peristome and other regions in *Stentor* and *Vorticella*, in other words the axial gradient in rate is less steep in *Paramœcium* than in those forms.

A general relation seems to exist between the character of the gradient and the degree of morphological and physiological "cephalization" in these different forms. In *Paramœcium* the gradient is comparatively slight and apparently uniform except for the vacuolar regions. In *Stylonychia* and *Euplotes* the gradient is steeper, *i. e.*, the differences are greater but here also it is uniform or nearly so. In *Stentor* a distinct gradient, steeper in the more anterior regions than that in *Paramœcium*, extends throughout the length of the body, but the peristome region is distinctly marked off from other parts by its higher rate, *i. e.*, the gradient from the peristome region to other parts is steeper than the gradient in other regions. And finally in *Vorticella* and *Carchesium* the gradient between peristome and other regions is steep as in *Stentor* but in other parts of the body the gradient is slight. When we compare these facts with the differences in morphology and behavior of these different species and of the different regions of the body in each the general parallelism between the different groups of facts is strikingly evident. The degree of morphological and physiological "cephalization" in these infusoria parallels the degree of difference between the rate of metabolism in the apical region and that in other regions. The obvious inference from the facts is that the axial metabolic gradient has some very intimate relation to the morphological and physiological polarity of these organisms.

The occasional departures from the general rule such as the case observed in *Stentor* where disintegration in the posterior half of the body proceeded from the posterior end and the rare cases in *Paramœcium* where a swelling appears over the posterior vacuole only undoubtedly result from temporary stimulation of the posterior regions. The dynamic gradient is not to be conceived as fixed and invariable; it may be temporarily or in many cases even permanently eliminated. But wherever it is present to a certain degree and more or less continuously existent it must determine both morphological and physiological differences along the axis.

The facts of embryology indicate that a gradient in rate of metabolism exists or arises early in the embryonic development of every animal and that the region of highest rate becomes the apical or cephalic region. In plants likewise the apical region of the axis is undoubtedly the region of highest metabolic rate.

Attention has already been called in earlier papers (Child, '13*a*, '13*c*) to these and to other facts and further data will be presented in the future. The facts indicate as I believe that a gradient in the rate of metabolism or of certain metabolic processes is the dynamic basis of morphological and physiological polarity in organisms. The existence of such a gradient in unicellular organisms and the striking relation between its features and the morphological and physiological features of the different species in these as well as in multicellular forms is to say the least suggestive. It points very clearly to a fundamental similarity between unicellular and multicellular organization and differentiation.

These observations are also of some interest in another connection. Loeb ('99) has advanced the hypothesis that the nucleus is the chief organ of oxidation in the cell and has interpreted the inability of non-nucleated pieces or cells to regenerate to asphyxiation in the absence of the nucleus. Verworn ('91, '04), on the other hand, has shown that non-nucleated pieces of various ciliates are as susceptible to lack of oxygen and recover as readily when it is present as nucleated cells or pieces. These facts indicate that respiration takes place in the cytoplasm, even when the nucleus is absent. Later investigations by various authors support and confirm Verworn's view.

My own observations show that the nucleus is much less susceptible to cyanide than the cytoplasm. If the cyanides inhibit in some way the oxidation processes as most authorities believe the much greater susceptibility of the cytoplasm indicates that the oxidations are localized in it, at least to a much greater extent than in the nucleus. Moreover, the existence of a gradient in susceptibility to cyanide in the cytoplasm and particularly in the ectoplasm indicates that different rates of oxidation are localized in different regions of the unicellular body in very much the same way as in multicellular forms. But even if the cyanides produce their effect in some other way than by inhibiting the oxidation processes specifically, whether their action is upon some other reactions of the metabolic complex or upon the metabolic substratum of the cell, they make it evident that a dynamic gradient exists along the main axis of the unicellular ciliate infusorian body.

Summary.

1. An axial gradient in susceptibility to KCN is present in *Stentor*, *Stylonychia*, *Vorticella*, *Carchesium* and *Paramœcium*. This suceptibility gradient indicates the existence of a gradient in rate of metabolism or of oxidation processes.

2. In all forms examined the anterior or apical region is the highest point in the general gradient, although localized regions of still higher rate such as the vacuolar regions of *Paramœcium* may exist.

3. A close parallelism exists between the character of the gradient and the morphological and physiological features of the species. Where the gradient is uniform and slight, structural and physiological differences along the axis are slight and where

the changes in steepness are great, marked morphological and physiological differences are present.

4. The facts indicate that the axial gradient is the dynamic basis of morphological and physiological polarity in the ciliate infusoria as well as in other organisms.

REFERENCES.

Child, C. M.

'12 Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction, IV. Certain Dynamic Factors in the Regulatory Morphogenesis of *Planaria dorotocephala* in Relation to the Axial Gradient. Journ. Exp. Zool., Vol. XIII., No. 1, 1912.

'13a Certain Dynamic Factors in Experimental Reproduction and their Significance for the Problems of Reproduction and Development. Arch. f. Entwickelungsmech., Bd. XXXV., H. 4, 1913.

'13b Studies, etc., V. The Relation between Resistance to Depressing Agents and Rate of Metabolism in *Planaria dorotocephala* and its Value as a Method of Investigation. Journ. Exp. Zool., Vol. XIV., No. 2, 1913.

'13c Studies, etc., VI. The Nature of the Axial Gradients in *Planaria* and their Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., Bd. XXXVII., H. 1, 1913.

Loeb, J.

'99 Warum ist die Regeneration kernloser Protoplasmastücke unmöglich oder erschwert? Arch. f. Entwickelungsmech., Bd. VIII., H, 4, 1899.

Verworn, M.

'01 Die Physiologische Bedeutung des Zellkerns. Arch. f. d. ges. Physiol., Bd. LI., 1891.

'04 Die Lokalisation der Atmung in der Zelle. Festschr. zum siebsigsten Geburtstag von Ernst Haeckel. Jena, 1904.